been added, was concentrated to a small volume. Paper chromatography of the concentrate in toluene–propylene glycol showed two components when the papers were developed according to Rydon and Smith. Chromatography over 300 g. of Florisil and elution with ether afforded two crystalline fractions, 8 and 9, which were homogeneous and identical with the less polar spot noted in the paper chromatogram of the mixture. Recrystallization from acetone–ether–hexane gave 0.271 g. of I (L-proline-L-leucinediketopiperazine), m.p. 160–162°; $[\alpha]_D^{25}$ −120° (dioxane), λ_{max}^{Nujol} 3.06 μ (N—H), 5.95 μ and 6.08 μ (amide carbonyl).

Anal. Calcd. for $C_{11}H_{18}O_2N_2$: C, 62.83; H, 8.62; N, 13.33; mol. wt. 210. Found: C, 63.11; H, 8.83; N, 13.84; mol. wt. (Rast) 221.

Fractions 10–16 (0.983 g.) eluted with ether were mixtures of I and II. Fractions 17–20 (0.557 g.) also eluted with ether, were predominantly II. The latter fractions were pooled and partitioned on 300 g. of Chromosorb W which held 300 g. of propylene glycol saturated with toluene. The pool of fractions 17–20 was placed on the partition column in the manner noted previously for steroid mixtures and eluted with toluene saturated with propylene glycol. Fractions 30–35 contained only the more polar component and these were pooled and crystallized from acetone–ether–hexane affording 0.075 g. of II (L-proline-L-valinediketopiperazine), m.p. 189–190°, $[\alpha]_D^{25}$ −164.5° (dioxane), λ_{max}^{Nujol} 3.06 μ (N—H), 5.96 μ and 6.12 μ (amide carbonyl).

Anal. Calcd. for $C_{10}H_{16}O_2N_2$: C, 61.45; H, 8.21; N, 14.3; mol. wt. 196. Found: C, 61.70; H, 8.33; N, 14.29; mol. wt. (Rast) 225.

Hydrochloric acid hydrolyses of I *and* II. A sample of 0.5 mg. of I was hydrolyzed with 1.0 ml. of 6*N* hydrochloric acid at 110° in a sealed tube for 24 hr. The hydrolysis mixture was concentrated to a residue which was dissolved in citrate buffer and analyzed according to Moore, Spackman and Stein[4] in a Phoenix Precision Instrument Co. (Phila., Pa.), Model K-5000 amino acid analyzer. Proline (0.27 mg.) and leucine (0.28 mg.) were identified by their elution volumes which were compared with authentic controls. Identities were confirmed by conversion to the dinitrophenyl derivatives which were prepared and characterized by paper chromatography according to Biserte.[13]

By the same procedure II (0.65 mg.) afforded proline (0.32 mg.) and valine (0.34 mg.) identified in the same way.

Optical forms of the amino acids. Samples of I and II were hydrolyzed according to the method of the preceding experiment and freed of hydrochloric acid by repeated concentration of the aqueous solutions. The residues were submitted to the action of D-amino acid oxidase (Worthington Biochemical) in the Warburg apparatus at 37°.[14] The results are recorded in Table I.

TABLE I

Substrate	Amount	Oxygen Consumed
DL-Leucine	15.3 μM	3.96 μM
DL-Valine	17.1 μM	4.67 μM
DL-Proline	17.4 μM	3.16 μM
I (hydrolyzed)	8.9 μM	0.27 μM
II (hydrolyzed)	9.7 μM	0.10 μM
Blank		±0.30 μM

BLOOMFIELD, N. J.

(13) G. Biserte and R. Osteux, *Bull. Soc. Chim. Biol.*, **33**, 50 (1951).

(14) S. P. Colowick and N. O. Kaplan, *Methods in Enzymology*, II, Academic Press, New York, 1955, p. 199.

Phenoxazines III. Dialkylaminoalkylphenoxazine-10 Carboxylates

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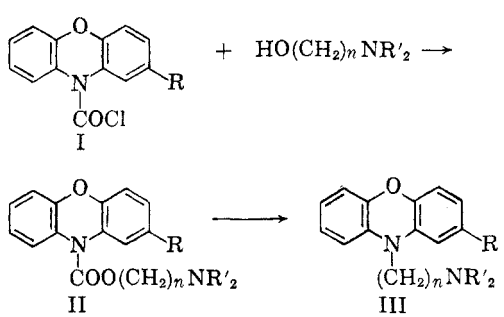
Various dialkylaminoalkyl esters of phenoxazine-10-carboxylic acid (II) were prepared by the reaction of the appropriate amino alcohol with phenoxazine-10-carbonyl chloride (I). This acid chloride was obtained by treating a toluene solution of phenoxazine with phosgene, a method which has already been used in the phenothiazine and carbazole series.[1]

These esters were decarboxylated by heating and the corresponding dialkylaminoalkylphenoxazines (III) were obtained.[2] An ester of 2-ethylphenoxazine-10-carboxylic acid (II. R = C_2H_5) was also treated in the same way. The aminoalkylphenoxazines (III) were identical with those prepared by other methods.[3]



EXPERIMENTAL[4]

Phenoxazine-10-carbonyl chloride (I. R = H). A 30% (w/w) solution (40 g.) of phosgene in toluene was added to a suspension of 11 g. of phenoxazine in 25 ml. of dry toluene in a Iena autoclave. The mixture was heated in an oil bath at 115° for 3 hr., under agitation with a magnetic stirrer. After cooling, the solution was evaporated to dryness under reduced pressure and the residue was crystallized in ethyl acetate. There was obtained 13.9 g. (94%), m.p. 139–141°.

3'-Dimethylaminopropylphenoxazine-10-carboxylate hydrochloride (II. R = H; $NR'_2 = N(CH_3)_2$; $n = 3$). A mixture of 7.5 g. (0.03 mole) of phenoxazine-10-carbonyl chloride and 3.15 g. (0.03 mole) of 3-dimethylaminopropanol in 30 ml. of dry benzene was heated on the steam bath for 17 hr.

(1) R. Dahlbom, *Acta Chem. Scand.*, **7**, 879 (1953); A. W. Weston, R. W. Denet, and R. J. Michaels, *J. Am. Chem. Soc.*, **75**, 4006 (1953).

(2) This method was described for various phenothiazine- and carbazolecarboxylates: J. Schmitt, J. Boitard, P. Comoy, A. Hallot, and M. Suquet, *Bull. Soc. Chim. France*, 938 (1957); B.A.S.F. (H. Friederich, D. A. Grosskinsky, and A. Amann), German Pat. **939,630** (1956); *Chem. Abstr.*, **53**, 8172 (1959); Bayer, Brit. Pat. **808,049** (1959); *Chem. Abstr.*, **53**, 12312 (1959).

(3) H. Vanderhaeghe, and L. Verlooy, *J. Org. Chem.*, **26**, 3827 (1961).

(4) All melting points are uncorrected. The microanalyses were performed by Dr. A. Bernhardt, Mülheim (Ruhr) Germany.

TABLE I

DIALKYLAMINOALKYLPHENOXAZINE-10-CARBOXYLATES (II. R = H)

$(CH_2)_nNR_2'$	M.P.° Dec. of Salt	Formula	Nitrogen Calcd.	Nitrogen Found
$CH_2CH_2N(CH_3)_2$	196–197	$C_{17}H_{18}N_2O_3 \cdot HCl$	8.36	8.21
$CH_2CH_2N(C_2H_5)_2$	132–134	$C_{19}H_{22}N_2O_3 \cdot HCl$	7.72	7.74
CH_2CH_2N⟨ ⟩	170–172	$C_{19}H_{20}N_2O_3 \cdot HCl$	7.76	7.71
$CH_2CH_2CH_2N(CH_3)_2$	215–216	$C_{18}H_{21}N_2O_3 \cdot HCl$	8.03	8.23
$CH_2CH_2CH_2N(C_2H_5)_2$	184–186	$C_{20}H_{24}N_2O_3 \cdot HCl$	7.43	7.30

After cooling, the precipitate was filtered off and dried, 7.2 g., m.p. 198–200° dec. By recrystallization in absolute ethanol 6.30 g. (60%) of white crystals, m.p. 215–216° dec. was obtained.

2-Ethyl-10-(3'-N-pyrrolidinopropyl)phenoxazine hydrochloride (III. R = C_2H_5; NR'_2 = N⟨ ⟩; n = 3). (a) *2-Ethylphenoxazine-10-carbonyl chloride.* A 34% (w./w.) solution (20 ml.) of phosgene in toluene was heated in a closed reaction vessel with 6.76 g. (0.032 mole) of 2-ethylphenoxazine in 10 ml. of toluene at 75° for 3.5 hr. The solution was evaporated to dryness and the oil (8.75 g., 100%) did not solidify after being kept in a refrigerator for several days.

(b) *Preparation and decarboxylation of 2-ethylphenoxazine-10-carboxylic acid 3'-N-pyrrolidinopropyl ester.* A solution of 8.75 g. (0.032 mole) of 2-ethylphenoxazine-10-carbonyl chloride and of 9.1 g. (0.070 mole) of 2-pyrrolidinopropanol in 30 ml. of dry benzene was heated on the steam bath for 5.5 hr. After cooling, the mixture was diluted with the same volume of ether and extracted with 60 ml. of 5% hydrochloric acid. After separation the aqueous phase was made alkaline and again extracted with ether. After evaporation of the ether, the residue was decarboxylated by heating in oil bath at 220–230° under reduced pressure (20–40 mm.). When the evolution of carbon dioxide subsided, the product was distilled *in vacuo* to give 7.3 g. (70%) of an oil, b.p. 215°/0.8 mm. The base was dissolved in a solution of hydrochloric acid in absolute ethanol and yielded 6.45 g. of III, m.p. 174–175°, identical with the same product prepared by other methods.[3]

10-(3'-Dimethylaminopropyl)phenoxazine hydrochloride (III R = H, NR_2' = $N(CH_3)_2$; *n* = 3) The hydrochloride of the carboxylate (6.25 g.) described above was dissolved in water and extracted with ether, after having been made alkaline. The residue obtained after evaporation of the ether was decarboxylated at a temperature of 215° under reduced pressure (35–45 mm.). The residual oil was distilled *in vacuo* (173°/0.3 mm.) and transformed into the hydrochloride to give 2.73 g. of white crystals, m.p. 132–134°, identical with the product prepared by another method.[3]

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Reaction of Chlorinated Urea Products with Ammonia and Ethylamine

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As *p*-urazine was needed in studies initiated in this laboratory[1] its synthesis was attempted by a procedure essentially similar to a method described by Chattaway[2] in which a chlorinated urea product is treated with aqueous ammonia. Many repeated attempts to prepare *p*-urazine by this method produced a substance that appears to be biurea,[3] as it exhibited properties identical to a sample obtained from Eastman Kodak Co. and one prepared by the action of urea on hydrazine hydrate,[4] and unlike a sample of *p*-urazine prepared by the method of Curtius and Heidenreich[4] in which the ethyl ester of hydrazinedicarboxylic acid is heated with hydrazine hydrate. Like biurea, the aqueous solution of the product obtained by ammoniating the chlorourea product was neutral. It gave no color with ferric chloride but gave an immediate positive test with Fehling's solution. *p*-Urazine dissolves in water to give an acid solution, gives a red color with ferric chloride, and is supposed to give a positive test with Fehling's solution only upon extended heating. Authentic biurea and the ammoniated chlorourea product melted at 246–258°, whereas *p*-urazine is listed as melting at 260°.[4] The ammoniated chlorourea product gave no noticeable depression of the melting point when mixed with an authentic sample of biurea but gave a definite depression when mixed with an authentic sample of *p*-urazine.

An X-ray examination of biurea and *p*-urazine was performed by Dr. Virginia Russell of the Chemistry Department at Syracuse University.[5] A well formed crystal of biurea was mounted and x-ray pictures were taken on a Weissenberg camera. The crystal was examined with the rotation axis parallel to a diagonal (not 90° apart).

Rotation patterns of an authentic biurea sample and the ammoniated chlorourea product showed two-fold rotation in each. A Weissenberg picture of a zero layer indicates two axes which were found at an angle of 62° to each other. From this evi-

(1) The support of these studies by W. R. Grace & Co., Clarksville, Md., is gratefully acknowledged.

(2) F. D. Chattaway, *J. Chem. Soc.*, **95**, 235 (1909).

(3) Biurea is also known as hydrazodicarbonamide, carbaminyl hydrazide, hydrazine-*N*,*N'*-dicarboxylic acid amide, and hydrazoformamide.

(4) T. Curtius and K. Heidenreich, *J. prakt. Chemie*, (2) **52**, 454 (1895).

(5) Present address: General Electric Co., Electronics Park, Syracuse 1, N. Y.